REMARKS/ARGUMENTS

The Pending Claims

Claims 36, 37, and 40-70 are pending and are directed to a bacterial artificial chromosome (BAC) and a method of producing and mutagenizing same.

The Office Action

Claims 36, 37, and 40-70 remain rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Messerle et al., *J. Mol. Med.*, *74*: B8 (1996) ("the Messerle reference") and Chartier et al., *J. Virol.*, *70*: 4805-4810 (1996) ("the Chartier reference") alone or in combination with one or more of the following secondary references: Ehtisham et al., *J. Virol.*, *67*: 5247-5252 (1993) ("the Ehtisham reference"), Gage et al., *J. Virol.*, *66*: 5509-5515 (1992) ("the Gage reference"), Chen et al., *Mol. Cell. Biol.*, *7*: 2745-2752 (1987) ("the Chen reference"), and Luckow et al., *J. Virol.*, *67*: 4566-4579 (1993) ("the Luckow reference"). Reconsideration of these rejections is respectfully requested.

Discussion of Rejections Under 35 U.S.C. § 103

Claims 36, 37, and 40-70 have been rejected under Section 103 as allegedly unpatentable over the combination of the Messerle reference and the Chartier reference alone or in combination with one or more secondary references. This rejection is traversed for the reasons set forth below.

For subject matter defined by a claim to be considered obvious, the Office must demonstrate that the differences between the claimed subject matter and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. § 103(a); see also *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). The ultimate determination of whether an invention is or is not obvious is based on certain factual inquiries including: (1) the scope and content of the prior art, (2) the level of ordinary skill in the prior art, (3) the differences between the claimed invention and the prior art, and (4) objective evidence of nonobviousness. *Graham*, 383 U.S. at 17-18, 148 U.S.P.Q. at 467.

Consideration of the aforementioned Graham factors here indicates that the present invention, as defined by the pending claims, is unobvious in view of the cited references.

Regarding the scope and content of the prior art, the Messerle reference discloses an attempt to clone a full length MCMV genome (about 235 kb) as a BAC in *E. coli*. Specifically, the Messerle reference discloses the generation of a recombinant MCMV virus containing a BAC-vector integrated into the MCMV genome. The circularized or linear recombinant virus was electroporated in *E. coli* strain DH10B. Both of the resulting plasmids contain deletions in the conserved region of about 15 kb. Therefore, none of the resulting BAC-plasmids comprises all the genes that are essential for the generation of an infectious virus, as required by the pending claims. This is further evidenced by the fact that transfection of each of the BAC plasmids alone did not result in any infectious progeny. Rather, only the co-transfection of both plasmids led to plaque formation due to complementation between the deficient BAC plasmids.

The Office Action states that the Messerle reference discloses cloning of a full length MCMV (see Office Action at page 8, lines 4-6). Applicants respectfully disagree with the Office Action in this regard, as the Messerle reference discloses that the BAC-plasmids lack 15 kb of essential sequence.

The Chartier reference discloses the assembly of an entire adenovirus genome via plasmid recombination in *E. coli*.

For the sake of argument and for purposes of the present analysis, one of ordinary skill in the art can be assumed to be someone with an advanced degree and a few years of experience in the relevant art.

With respect to the differences between the claimed invention and the cited references, Applicants note that the Messerle reference does not disclose a BAC containing all genes essential for the generation of an infectious herpes virus in a host cell, wherein the host cell does not contain any herpes virus genes or herpes virus proteins. The Chartier reference differs from the claimed invention in several respects. First, the Chartier reference discloses plasmid recombination in *E. coli*, while the claimed BAC is produced by recombination in eukaryotic cells.

Applicants submit that one of ordinary skill in the art would not be motivated to combine the disclosures of the Messerle reference and the Chartier reference, as evidenced by the fact that such a combination was never made, despite the Chartier reference being cited over 90 times since its initial publication. None of the publications which cite the Chartier reference discloses the production of infectious viruses greater than 100 kb.

The Examiner maintained his objections from the last Office Action because he is of the opinion that the Chartier reference would disclose a method suitable for generating the inventive BAC containing an infectious herpes virus genomic sequence larger than 100 kb and containing all genes that are essential for the generation of an infectious herpes virus in a host cell that does not contain herpes virus genes or proteins (referred to hereinafter as "infectious herpes BAC"). The Applicants respectfully disagree that the technique of Chartier is suitable for the generation of an infectious herpes BAC. Furthermore, the teachings of the Messerle reference and the Chartier reference were not combined in the present invention as they are incompatible with each other for the reasons set forth in the Declaration under 37 C.F.R. § 1.132 of Martin Messerle. Moreover, even if one of ordinary skill in the art were motivated to combine the disclosures of the Messerle reference and the Chartier reference, the combination does not provide a solution to the problem.

The scientific reasons why the Chartier reference does not allow the production of infectious BACs were previously outlined in detail. In the Office Action, the Examiner maintains that the Chartier reference would be suitable for the generation of infectious herpesvirus BACs. However, the Office Action fails to identify which adaptations of the technique should be performed other than mentioning that selecting a different *E. coli* strain would have been obvious. The Office Action fails to clarify which adaptations will have to be made to counter the following problems:

(i) The genome structure of herpes viruses is complex in that it contains numerous direct and indirect sequence-repeats (see application at page 24, Example 4, "The genome of the MHV68 contains a variable number of terminal repeats at its ends" (TR; Fig. 10, top).". The Chartier reference found that the pTG3602 construct exhibited a remarkable stability due to the insertion of the 2kb ppolyII sequence between the two viral ITRs, avoiding the generation of palindromic sequences (Chartier reference at page 4806, right column, last

sentence of 1st paragraph). In view of the multitude of direct and indirect repeats of the herpes virus genome, one of ordinary skill in the art would expect that a herpes virus genome contained in a BAC would not be supported by the *E. coli* recBC sbcBC strain used by the Chartier reference. Thus, one of ordinary skill in the art would understand that the technique described in the Chartier reference is not suitable for the assembly of a herpes virus genome.

(ii) Furthermore, most herpes viruses (HCMV, HSV, etc.) contain an identical terminal repeat, the so-called a-sequence, at the respective ends of the genome. This sequence is essential for the cleavage and packaging of the viral genome. If this sequence would have been included in a region used for a recombination event, like it was performed in the Chartier reference for the Ad5 genome, a skilled person would expect that cloning of only the a-sequence would occur, but not of the entire genome. In addition, the a-sequence occurs multiple times in the rest of the genome (within the internal repeat). One of ordinary skill in the art would expect from the multiple occurrences of the a-sequence that the cloned genome would be unstable (as outlined in section (i)), or that only a subfragment of the genome could be obtained. Therefore, the technique described in the Chartier reference is useful for repetitive sequences. Herpes virus genomes contain multiple repetitive sequences, some of which are essential to viral function and can not be omitted. Hence, one can not keep them in view of the aforementioned limitations of the Chartier reference and one can not omit them.

(iii) The a-sequences are flanked by additional repetitive sequences: the b- and c-repeats Thus, an extension of the region that may be used for a homologous recombination event (that requires unique sequences to be accurate) is not available for compensating any of the defects set forth in (i) and (ii). The Chartier reference teaches to use approximately 1 kb of unique sequence at the respective ends of the genome to be cloned (i.e., regions of the plasmid shown in white boxes of Figure 1A, top). Such regions of unique terminal sequences are not readily available for herpes viruses.

See, e.g., *Fields Virology 3rd Ed.* (1996), Ed. Fields, Knipe, Howley, publ. Lippincott, Wiliams & Wilkins; therein the essay of Bernard Roizman, chapt. 71, "The Family Herpesviridae: A brief introduction" and the essay of Bernard Roizman, Amy E. Spears, chapt. 72 "Herpes Simplex Viruses and Their Replication."

(iv) The *E. coli* strain used in the Chartier reference is not suitable for carrying out the present invention, because the technique of the Chartier reference requires that *E. coli* is transformed with a linear virus genome. The strain of the Chartier reference is not defective in *E. coli's* endogenous restriction endonuclease system. Transformation of the Chartier strain with a linear genome of a size of a Herpes virus, which is three times the size of the Ad5 genome, would therefore be expected to lead to fragmentation of the genome through the action of the restriction endonuclease system because of numerous (if not multiple) occurrences of restriction sites that are subject to cleavage by *E. coli's* endonuclease restriction enzyme systems.

(v) The necessity of the Chartier reference to transform a linear virus genome into *E. coli* is indicative of a number of other complications:

(a) The handling of linear DNA fragments greater than 100kb is difficult and prone to single- and double-strand breakages of the DNA. It is uncertain whether the infectious linear herpes virus DNA could be produced in sufficient quality to transform *E. coli*.

(b) It is furthermore highly uncertain, that a linear DNA greater than 100kb can be efficiently transformed into *E. coli*, as acknowledged by the Chartier reference with respect to transformation of the Ad5 genome.

Indeed, the Chartier reference itself acknowledges certain limitations of the disclosed method for generating infectious viruses comprising large genomes when it states:

> "*the size of the Ad5 genome (36 kb) constitutes a limiting factor for the efficiency of transformation*. This possibility is supported by the observation that *E. coli* transformation is inhibited when increased amounts of Ad5 DNA are used (Table 1)"

(paragraph bridging pages 4805-4806, emphasis added). Thus, techniques that may be used to generate adenoviral genomes, and possibly other viral genomes of comparable size, may no necessarily be used to generate herpes viruses larger than 100 kb for the reasons of record.

In the Chartier reference, recombination occurs between a specially designed plasmid that contains Ad5 DNA fragments. These are the only sequences of sufficient homology in

the entire system to allow for recombination in the *E. coli* strain used in Chartier. As argued previously, herpes virus contains multiple regions that would be recognized by such an *E. coli* strain, which would result in recombinant herpes virus BAC fragments that do not contain all genes that are essential for the generation of an infectious herpes virus.

What is essential is that an *E. coli* strain capable of recombining adenoviral DNA is not suitable to recombine herpes virus DNA. The *stability* of a generated infectious BAC of >100 kb is not relevant. Rather, what is relevant is the fact that *E. coli* is incapable of generating such an infectious herpes BAC. It is not relevant what strain to take for the *maintenance* of the generated infectious BACs. The Office Action's contention that the technique disclosed in the Chartier reference is not sensitive towards the plasmids used also is not relevant to the presently claimed invention, since it is sensitive towards having a unique homologous sequence, which is available in Ad5, but not for herpes virus. In other words, the technique of the Chartier reference may employ a BAC, but it is not suitable for herpes virus, irrespective of what vector is used, and irrespective of what further adaptations are made to the *E. coli* strain employed.

The fact that E. coli is not suitable to produce the required recombinants is further evidenced by the Declaration under 37 C.F.R. § 1.132 of Ulrich H. Koszinowski, which describes experiments demonstrating that the method disclosed in the Chartier reference cannot be used to generate virus genomes that are larger than an adenovirus genome (i.e., 36 kilobases), such as a herpes virus genome.

The Office Action argues that Kong et al., *J. Virol. Methods*, *80*: 129-136 (1999), demonstrates that the *E. coli* strain disclosed in the Chartier reference is capable of generating infectious herpes virus genomes. According to the Office Action, the Kong reference discloses an *in vivo* recombination cloning procedure that supports the routine manipulation of large DNAs, such as two cosmids comprising overlapping HSV-1 DNA fragments to generate plasmids up to 65 kb. Applicants respectfully disagree with the Office Action's interpretation of the Kong reference. In this regard, the Kong reference does not employ the technique disclosed in the Chartier reference. Specifically, Chartier requires the transformation of one single linear DNA of 35 kb together with a linearized vector, which vector is modified to allow recombination. The Kong reference employs multiple (i.e., up to

six) overlapping DNA fragments (cosmids not plasmids), and a specifically adapted transformation protocol that is different than that disclosed in the Chartier reference.

Notwithstanding these clear differences, the Kong reference also does not disclose the generation of an infectious BAC that is greater than 100 kb, as required by the pending claims. Indeed, the Kong reference acknowledges that the method disclosed therein can be used for "the construction of plasmids *up to 65 kb in size*" (see abstract). Additionally, the Kong reference in fact proves that the Office Action's allegation that the technique of the Chartier reference is *suitable* for the construction of such large viral genomes is incorrect. For example, Page 134, 1st column of the Kong reference states:

> "*Attempts to use an analogous cloning strategy to construct an ~ 80 kb plasmid, containing the HSV-1 inserts from three cosmids, were not successful (not shown).*"

It is also respectfully pointed out that the Kong reference was published after the present invention was made. The fact that Kong needed to adapt the transformation protocol of *E. coli* for large cosmids in 1999 also indicates that, as of the filing date of the present application, the transformation of a linear herpes virus genome greater than 100 kb required modifications that were not known in the art (e.g. at least those modification of Kong, but more likely even further modifications that are not known even as of today).

Therefore, the Kong reference does not evidence the feasibility of using the Chartier reference to generate infectious herpes virus genomes because the Kong reference requires further adaptation of the Chartier reference, including the use of cosmids instead of BACs, and constructing genomes up to 65kb instead of 100 kb or greater. None of these adaptations yields an infectious virus vector.

Thus, in view of the foregoing, in order to initiate a recombination event in the E.coli strain disclosed in the Chartier reference, one of ordinary skill in the art would need to: (i) adapt the vector that should "host" the final product with appropriate ends, (ii) provide a linear DNA with appropriate ends that (a) overlap those ends of the vector and (b) that are not contained in the remainder of the linear DNA, and (iii) transform the linearized vector and the linearized DNA into the *E. coli* with an appropriate efficiency. As discussed above, one of ordinary skill in the art would not expect to obtain a linear DNA fragment of at least 100 kb using *E. coli* due to (i) the known size limitations of transformation, (ii) the *E. coli* strain

disclosed in the Chartier reference having a large DNA fragment unrestricted by its endonucleases, (iii) the *E. coli* strain disclosed in the Chartier reference not recombining these BACs due to the lack of appropriate linear homologous terminal sequence portions, which need to occur only at the termini and not internally in essential genome regions, and (iv) the presence of various internal repeats which would prevent the production of entire herpes virus BACs.

Applicants also remind the Office that [o]rdinarily, there must be some form of evidence in the record to support an assertion of common knowledge. See, e.g., *In re Lee*, 277 F.3d. 1344-45, 61 U.S.P.Q. 2d 1434-35 (Fed. Cir. 2002); I*n re Zurko*, 258 F.3d 1379, 59 U.S.P.Q. 2d 1693 (Fed. Cir. 2001) (holding that general conclusions concerning what is "basic knowledge" or "common sense" to one of ordinary skill in the art without specific factual findings and some concrete evidence in the record to support these findings will not support an obviousness rejection); see also M.P.E.P. § 2144.03. As discussed herein, the Examiner's contention that "the method of Chartier et al. is further applicable to any large virus genome" (Office Action at page 4, last paragraph) is simply untrue. There is nothing in any of the cited references to corroborate this statement, and the Office Action has provided no evidence to support this assertion.

None of the secondary references cited by the Office Action compensates for the deficiencies of the Messerle reference. In this respect, none of the Ehtisham, Gage, Chen, and Luckow references discloses or suggests a BAC containing all genes essential for the generation of an infectious herpes virus in a host cell, wherein the host cell does not contain any herpes virus genes or herpes virus proteins.

For purposes of considering the obviousness rejections, there is no need to consider any objective evidence of nonobviousness.

Considering all of the Graham factors together, it is clear that the present invention would not have been obvious to one of ordinary skill in the art at the relevant time in view of the combination of cited references. Accordingly, the obviousness rejections under Section 103 should be withdrawn.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned agent.

Respectfully submitted,

Melissa E. Kolom, Reg. No. 51,860
LEYDIG, VOIT & MAYER, LTD.
Two Prudential Plaza, Suite 4900
180 North Stetson Avenue
Chicago, Illinois 60601-6731
(312) 616-5600 (telephone)
(312) 616-5700 (facsimile)

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